BIOPESTICIDES REGISTRATION ACTION DOCUMENT

Coat Protein Gene of Plum Pox Virus

PC Code: 006354

U.S. Environmental Protection Agency
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division

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GLOSSARY OF ACRONYMS AND ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service (USDA)
BPPD	Biopesticides and Pollution Prevention Division
CAS	Chemical Abstracts Service
40 CFR	Title 40 of the Code of Federal Regulations
C5 or C5 plum	C5 HoneySweet Plum
CPG-PPV	Coat Protein Gene of Plum Pox Virus
cDNA	Copied (or Copy) DNA
dsRNA	Double-Stranded RNA
cos	Cosmid
^{o}C	Temperature in Centigrade or Celsius Degrees
DNA	Deoxyribonucleic Acid
EPA	Environmental Protection Agency (the "Agency")
ELISA	Enzyme-Linked Immunosorbent Assay
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
FR	Federal Register
g	Gram
IR4	Interregional Research Project Number 4
kg	kilogram
L	Liter
MRID No.	Master Record Identification Number
mg	Milligram
mL	Milliliter
µg	Microgram
MP	Manufacturing-Use Product
mRNA	Messenger RNA
NE	No Effect
NIOSH	National Institute for Occupational Safety and Health
OPP	Office of Pesticide Programs
OCSPP	Office of Chemical Safety and Pollution Prevention
PC	Pesticide Chemical
PCR	Polymerase Chain Reaction
PIP	Plant-Incorporated Protectant
PPV	Plum Pox Virus
PTGS	Post-Transcriptional Gene Silencing
PVCP	Plant Virus Coat Protein
RNA	Ribonucleic Acid
rRNA	Ribosomal RNA
TGAI	Technical Grade of the Active Ingredient
USDA	United States Department of Agriculture

I. OVERVIEW

On May 7, 2010, the Environmental Protection Agency (EPA, Agency) registered the pesticide product, C5 HoneySweet Plum, which contains the new plant-incorporated protectant (PIP) active ingredient, Coat Protein Gene of Plum Pox Virus (CPG-PPV). This PIP is a new active ingredient, and the registration is a new food use and the first outdoor use of the pesticide. Therefore, under the Agency's policy established October 1, 2009, to inform and provide the public an opportunity to comment on such registration decisions before they occur, there was a 30-day comment period on this action. All comments received, and the Agency's responses thereto, are included in the docket for this registration action (EPA-HQ-OPP-2008-0742).

This final decision on registration included Agency review and consideration of all public comments. The Agency believes that, based upon its assessment of the data and information submitted in support of the registration, it is in the best interest of the public and the environment to issue the registration. During the 30-day public comment period, EPA received 62 comments on the preliminary decision to register. The comments were informative, helpful, and served to assist us in our decision making.

A. Executive Summary

Plum Pox Virus (PPV) is a plant virus that reduces the quality of stone fruits, and eventually renders infected trees incapable of producing fruit. PPV was first described in Europe in 1915, where it is considered to be the most devastating viral disease of stone fruit. PPV is also present in the United States and Canada. Recent outbreaks in New York and Michigan underscore that PPV is becoming endemic despite containment efforts (bulldozing and disposal of infected vegetation, moratoria on the movement/transport of infected plant materials, and control of insect vectors).

PPV is an agricultural pest that causes significant economic losses to the stone fruit industry. The primary effects of the infection are reduction of fruit quality and crop yield. Stone fruits (including native or wild *Prunus* species) that are affected by PPV include plums, peaches, almonds, nectarines, apricots, and sweet and sour cherries. [http://www.apsnet.org/online/feature/PlumPox/plumpoxfs.pdf]. Various other non-*Prunus* dicotyledonous plants have been infected under experimental conditions [http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/00.057.0.01.054.htm].

When PPV infects a plant, its genetic material (a single strand of ribonucleic acid (RNA)) is inserted into the plant cells. This strand of RNA contains the genes needed to make new virions. One of these genes codes for the PPV coat protein – CPG-PPV. The infected cell makes viral coat protein in a similar manner as it produces its own plant proteins. RNA coding for the CPG-PPV are translated into the amino acid sequences that make up the protein. During virus replication, segments of double-stranded RNA are produced, but, ultimately, exact copies of the original single-stranded virus RNA chromosome are formed and packaged together with the coat proteins into new PPV virions. Small segments of double-stranded RNA (dsRNA) are formed during this process, and recognized by a defense mechanism within the host plant, post-

transcriptional gene silencing (PTGS). PTGS blocks the transcription as well as the production of viral proteins and RNA. This sequence of events results in the development of natural resistance to further PPV infections, but not before fruit degradation, leaf chlorosis, and other serious damage caused by the virus has occurred.

Uninfected plum trees can be genetically engineered to express the CPG-PPV. But, because the CPG-PPV is responsible for only one component needed for the production of new virions, these engineered plum trees cannot produce the virus. The U.S. Department of Agriculture, Agricultural Research Service-Appalachian Fruit Research Station (the Applicant) has developed a genetically engineered plum tree, called the C5 HoneySweet Plum (C5 or C5 plum), which expresses the CPG-PPV and is resistant to PPV infection. To create the C5 plum, the CPG-PPV is isolated and inserted into the plum genome as a transgene. During the plant's naturally occurring cellular processes, the transgenic CPG-PPV gene is transcribed. The messenger RNA (mRNA) copied from the inserted viral coat proteins genes forms abnormal regions of dsRNA, and the PTGS mechanism recognizes the abnormality and destroys segments with the same sequence. This process establishes the ability of the plant to respond quickly to a PPV infection, blocking the production of new virions and spread of the disease.

When purposely transferred to uninfected plants to make them resistant to PPV infection, the CPG-PPV is classified as a plant-incorporated protectant (PIP) and cannot be sold or distributed unless registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). All of the data requirements for registration of this PIP have been satisfied, except for the submission of data required from an independent laboratory validation of the applicant's analytical method that detects residues of the PIP deoxyribonucleic acid (DNA) in fresh and processed plum commodities for enforcement purposes. Insufficient time has elapsed since the requirement was imposed for the applicant to comply. In view of the clear benefits and minimal risks to human health and the environment, EPA has determined that the use of the CPG-PPV in C5 HoneySweet Plum, for the time it would take for the applicant to comply with the requirement, would not result in unreasonable adverse effects on the environment, and is issuing a time-limited registration of one year under FIFRA Section 3(c)(7)(C).

B. Use Profile

Active Ingredient:	Coat Protein Gene of Plum Pox Virus (CPG-PPV)
Office of Pesticide Programs (OPP) Chemical Code:	006354
Product Name:	C5 HoneySweet Plum (C5)
EPA File Symbol:	11312-I
Patent Number	US PP15,154 P2

Applicant/ Manufacturer:	U.S. Department of Agriculture, Agricultural Research Service-Appalachian Fruit Research Station 2217 Wiltshire Road, Kearneysville, WV 25430
Type of Pesticide:	Plant-Incorporated Protectant (PIP)
Use:	Stone Fruits and Almond
Target Pest:	Plum Pox Virus (PPV), a.k.a. Sharka disease

C. Regulatory Background

On October 29, 2008, EPA published a Notice of Receipt in the Federal Register (73 FR 64325), announcing that Interregional Research Project Number 4 (IR-4), Rutgers University, 500 College Rd. East, Suite 201 W, Princeton, NJ, 08540 (on behalf of the applicant, the United States Department of Agriculture, Agricultural Research Service-Appalachian Fruit Research Station) submitted an application to register a pesticide product containing a new active ingredient not included in any currently registered pesticide products. Four favorable comments were received during a 30-day comment period following the publication of this notice.

A petition (7E7231) seeking an exemption from the requirement of a tolerance for residues of the Coat Protein of Plum Pox Virus, in or on stone fruit and almond, was filed by IR-4 on behalf of the United States Department of Agriculture, Agricultural Research Service-Appalachian Fruit Research Station. The EPA published a Notice of Filing of the petition in the Federal Register on November 14, 2008 (73 FR 67512), and the public was given 30-day comment period. No comments were received. A final rule establishing the exemption from tolerance is codified under 40 CFR Part 174.

On April 1, 2010, EPA announced its preliminary decision to register the pesticide product, C5 HoneySweet Plum, containing the new PIP active ingredient, Coat Protein Gene of Plum Pox Virus (CPG-PPV). C5 HoneySweet Plum containing CPG-PPV is a new active ingredient, and the registration is a new food use and the first outdoor use of the pesticide. Therefore, consistent with EPA's public participation initiative for pesticide registration actions, we provided an opportunity for public comment prior to making the registration decision. The public comment period ended on April 30, 2010. We received 62 comments on this action. The comments and EPA's response document may be found in the docket for this registration action (EPA-HQ-OPP-2008-0742).

II. RISK ASSESSMENT SUMMARIES

Described below are summaries of EPA’s assessment of the product characterization, human health, and environmental risks from the use of the Coat Protein Gene of the Plum Pox Virus (CPG-PPV) as a plant-incorporated protectant. In its assessment, the EPA relied upon data and

other information submitted by the applicant. A more detailed description of the assessments can be found in the Appendices.

A. Product Characterization

The C5 HoneySweet Plum (C5) is one of a number of clones resulting from genetically engineering the CPG-PPV into the plum tree, *Prunus domestica* L. The C5 clone was selected for commercial development because of (1) its stability; (2) the durability of its resistance to PPV under a variety of environmental conditions, exposure to different virus strains and mixtures of virus strains; (3) the absence of coat protein production; and (4) the excellent quality of the fruit.

In field trials, C5 trees remained symptom-free following infection (either via aphid vectors or by grafting of infected plant tissues onto the trees). The genetic inserts into plum tree DNA were accomplished using an *Agrobacterium*-mediated transformation, and all aspects of the development of the C5 HoneySweet Plum trees have been published in peer-reviewed manuscripts (see "Application for Determination of Non-Regulatory Status for C5 ("HoneySweet") Plum (*Prunus domestica* L.) Resistant to Plum Pox Virus," http://www.aphis.usda.gov/brs/aphisdocs/04_26401p.pdf).

The mode of operation postulated for PPV resistance in C5 trees relies on the plant's natural PTGS defense mechanism. When the C5 DNA is being transcribed into messenger RNA (mRNA), the strand carrying the inserted DNA copy of the viral coat protein gene is also transcribed into mRNA. In some areas of the C5 DNA insert in the plant, mirror-image copies of the PPV-coat protein mRNA are produced that naturally bind each other, forming areas of dsRNA that the plant senses as abnormal and to which its host defenses react. The plant PTGS response to dsRNA corresponding to the PPV-coat protein produces shorter segments (approximately twenty-three nucleotide base pairs in length) of double-stranded RNA , and these segments are used as signals by the cell to initiate an immune-type response to any matching sequences of dsRNA in the plant. The plant's PTGS defense mechanism acts quickly to degrade matching dsRNA sequences and remains capable of destroying the viral genome if the plant becomes infected with PPV. Plant host defenses, once activated, also down-regulate expression of the DNA inserted into C5 so that the plant likely no longer expresses this gene and further transcription in RNA, and hence translation into coat proteins is not expected to occur.

For a more comprehensive discussion of the Agency's assessment of the product characterization data submitted in support of the registration of the CPG-PPV, refer to APPENDIX A.

B. Human Health Assessment

Human exposure to a variety of natural plant viruses and plant viral proteins is common in the diet, and exposure includes plant virus coat proteins (PVCPs). Plant viruses are not pathogenic to humans. A recent study (Zhang, et al., 2006) demonstrated that the human gastrointestinal tract harbors a wide variety of plant viruses within the intestines.

EPA reviewed the available scientific data and other relevant information submitted by the applicant in support of the registration of the PIP, CPG-PPV, and considered its validity, completeness and reliability, and the relationship of this information to human risk. EPA also considered the available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

The CPG-PPV is a PIP that consists of nucleic acids, and as such, is exempt from the requirement of a tolerance (40 CFR § 174.507). Although PPV coat protein generated from the PIP itself has not been detected in C5 plums, the Agency still considered whether there would be any dietary risks, especially toxicity, allergenicity, and anti-nutrient properties in the unlikely event that the coat protein is produced in the fruit. In its analysis, EPA considered the safety of dietary exposure to plant viruses and PVCPs, and the following three sections summarize the Agency's conclusions.

i. Plants infected with plant viruses have always been part of food supply without adverse effects.

Virus-infected food plants have always been a part of the human and domestic animal food supply, and components of plant viruses, including coat proteins, are often found in the produce of many types of crops. For example, at the beginning of this century, virtually every commercial cultivar of potatoes grown in the United States and Europe was infected with at least one potato virus. Even asymptomatic plants are often found to be infected. A common agricultural practice (since the 1920s) involves the intentional inoculation of healthy plants with a mild form of a virus in order to prevent infection by a more virulent form. To date, there have been no reports of adverse health effects in humans or animals associated with consumption of plant viruses in food.

Experiments have shown that viral coat protein levels expressed in plants that are genetically engineered to resist a virus infection can be 100-1000 times lower in concentration than in plants naturally infected by the virus. In the C5 plum, there is little to no detectable virus coat protein produced, since the inserted gene initiates the plant's natural defense mechanism before virus proteins can be manufactured.

ii. Plant viruses are not infectious to humans and animals.

Any virus/host relationship is characterized by a high degree of specificity. Plant viruses do not infect humans or other vertebrates, and usually only infect plants within a certain taxonomic group. In order to replicate, a plant virus usually relies on an insect or some other mechanical

vector to insert its genome through plant cell walls. Plant virus replication depends on either specific virus-encoded enzymes, or plant enzymes, to transcribe the viral genes, produce the viral components, and package them into new virus. Humans and other vertebrates lack the corresponding cellular "machinery" to transcribe, translate, and package plant viruses. Thus, it is reasonable to assume that a single component of PPV, the CPG-PPV, will not cause a viral infection or replication in humans and animals.

iii. Plant viruses are not toxic to humans and animals.

As stated previously, food from crops infected with plant viruses is (and has long been) consumed by humans and animals without any known toxicity or other adverse effects. Additional evidence of the lack of toxicity by a different route of exposure comes from the common practice of injecting laboratory animals with purified plant virus preparations to generate antibodies used for analytical tests (for example, to identify and confirm plant virus infections), without any adverse effects on the animals.

The Agency is not aware of any plant virus coat proteins that have been identified as a human food allergen. To determine whether the PPV coat protein would potentially cause toxicity or hypersensitivity, its amino acid structure was compared with known food allergens and toxins. No amino acid segments triggering a concern were identified.

The lack of production of PPV coat proteins by the C5 plum, together with reports indicating that there have been no hypersensitivity incidents or other adverse effects among researchers handling the trees, fruits, and other plant tissues since experimentation with C5 plum began in 1992, support the Agency's conclusion that, although unlikely to be produced in foods derived from C5 plum, PPV coat protein expressed in the C5 plum is safe for human dietary consumption.

For a more comprehensive discussion of the Agency's assessment of the data and information concerning human health submitted in support of the registration of the CPG-PPV, refer to APPENDIX B.

C. Environmental Effects Assessment

EPA considered all possible effects the CPG-PPV might have on mammalian, avian, fish, terrestrial and aquatic invertebrates, and plant non-target species. Since the PIP consists solely of a viral gene insert, and does not result in expressed viral coat proteins or produce new viruses, no adverse effects to species that interact with agriculture are expected.

For non-target plants, the Agency considered whether the gene could be transferred by hybridization of the C5 plum with other *Prunus* species. Successful gene flow in the environment, given the inefficiency of artificial intentional crosses from the hexaploid *Prunus domestica*, is considered highly unlikely. In the event hybridization occurred and the coat protein gene was transferred to a non-target plant, it is possible that any resulting resistance might be beneficial.

For a more comprehensive discussion of the Agency's assessment of the data and information concerning environmental risks submitted in support of the registration of the CPG-PPV, refer to APPENDIX C.

III. ENVIRONMENTAL JUSTICE

EPA seeks to achieve environmental justice, the fair treatment and meaningful involvement of all people, regardless of race, color, national origin, or income, in the development, implementation, and enforcement of environmental laws, regulations, and policies. To help address potential environmental justice issues, the Agency seeks information on any groups or segments of the population who, as a result of their location, cultural practices, or other factors, may have atypical, unusually high exposure to CPG-PPV compared to the general population. No comments advising the Agency of any sub-populations that may have atypical, unusually high exposure compared to the general population were received during the 30-day public comment period.

For additional information regarding environmental justice issues, please visit EPA's web site at http://www.epa.gov/compliance/environmentaljustice/index.html.

IV. BENEFITS AND PUBLIC INTEREST FINDING

All of the data requirements for the registration of the use of the CPG-PPV as a plant-incorporated protectant in C5 HoneySweet Plum have been satisfied, except for an independent laboratory validation of the Applicant's enforcement analytical method for the PIP DNA. Given that there has been insufficient time for the Applicant to comply with this requirement, EPA determined that a conditional registration, issued pursuant to FIFRA Section 3(c)(7)(C), is in the public interest.

EPA determines whether a conditional registration of a pesticide is in the public interest in accordance with the criteria set forth in the Federal Register dated March 5, 1986 (58 FR 7268). There is a presumption that registration of a pesticide is in the public interest if (1) the use is for a minor crop, (2) the use is a replacement for another pesticide that is of continuing concern to the Agency, (3) the use is one for which an emergency exemption under FIFRA section 18 has been granted (i.e., the basis for the exemption was lack of a registered alternative product), or (4) the use is against a pest of public health significance.

In 2009, the U.S. plum acreage (reported in CA, ID, MI, OR, and WA acres, representing approximately 95% of all acreage in the U.S., including plums and prunes) was 93,790 acres, well-below the 300,000 acre maximum for defining a minor/specialty crop (http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-01-22-2010_revision.pdf). Ninety-nine percent of the U.S. supply of dried plums is produced in California, and that state exports 46% of its production, which (although a minor crop) represents 70% of the world supply. Since the aphid vectors of the disease are common throughout the United States, the occurrence of PPV in this major plum-producing area could devastate the

industry and affect world supplies of product. This devastation has been the case in countries where PPV has already spread. Plum Pox is classified as an invasive species in the United States because of the significant economic losses that result to the orchard industry. If eradication of PPV was achieved in the U.S., this minor crop would remain vulnerable to reintroduction as long as the virus exists elsewhere.

Additionally, many *Prunus* species have ornamental value, and many of these are susceptible to PPV. Spread of the disease into high value ornamental nursery stock could cause significant losses to the nursery trade and its clients. Furthermore, wild species of *Prunus* provide food and shelter to wildlife, and natural habitat could be disrupted should those wild stone fruit species become infected.

Current control practices include the use of chemical pesticides to control aphids, which transmit (vector) the virus to the plants. Aphid infestations can be difficult to manage, so repeated applications of pesticides are usually necessary. Often, infected orchards must be destroyed, resulting in further economic losses. In Europe, over 100 million trees have been lost to this devastating virus.

C5 HoneySweet Plum offers several benefits, including better crop production (yield) in a minor/specialty crop, high quality fruit, and a reduction in the amount of chemical pesticides needed to control aphids that are vectors for PPV. Control of PPV via C5 HoneySweet Plum may contribute to protecting U.S. agriculture, as well as our managed landscapes and natural areas. Based on this information, EPA determined this conditional registration to be in the public interest.

V. RISK MANAGEMENT AND PROPOSED REGISTRATION DECISION

A. Determination of Eligibility

EPA is issuing a conditional registration for the CPG-PPV, expressed in C5 HoneySweet Plum. Pursuant to FIFRA Section 3(c)(7)(C), EPA may conditionally register a new pesticide active ingredient for a period of time reasonably sufficient for the generation and submission of required data that are lacking because insufficient time has elapsed since the imposition of the requirement for those data to be developed. EPA may grant such conditional registration only if EPA determines that (1) the use of the pesticide product during the period of the conditional registration will not cause any unreasonable adverse effect on the environment, and (2) the registration and use of the pesticide during the conditional registration is in the public interest. EPA believes these criteria have been met.

EPA concludes that insufficient time has elapsed since the imposition of the requirement for the applicant to submit data from an independent lab validation of the enforcement analytical method for the detection of the CPG-PPV in fresh or processed plum commodities. The first criterion is met, because the applicant provided sufficient data and other relevant information for EPA to determine that the use of the CPG-PPV in C5 HoneySweet Plum, during the period of the

conditional registration (one year), will not result in unreasonable adverse effects on the environment. The second criterion is met, because the use of the PIP in the C5 plum is in the public interest, as described in section IV above.

B. Regulatory Decision

On October 1, 2009, EPA announced a new policy to provide a more meaningful opportunity for the public to participate on major registration decisions before they occur. According to this new policy, EPA intends to provide a public comment period prior to making a registration decision for, at minimum, the following types of applications: new active ingredients; first food use; first outdoor use; first residential use; and other actions for which the Agency believes there will be significant public interest. Accordingly, EPA's preliminary decision to register this pesticide product was subject to a 30-day comment period (April 1, 2010 to April 30, 2010) as a new active ingredient with both first food and outdoor uses. EPA received 62 comments on its preliminary decision. The comments and EPA's response document may be found in the docket for this registration action (EPA-HQ-OPP-2008-0742).

The applicant provided sufficient data and other relevant information for EPA to determine that conditional registration of the CPG-PPV in C5 HoneySweet Plum under FIFRA 3(c)(7)(C) for one year will not result in unreasonable adverse effects on the environment. The human health and non-target organism data requirements are satisfied for the period of the conditional registration. EPA is imposing the requirement for the applicant to submit data from an independent lab validation of its enforcement analytical method as a condition of registration. Therefore, EPA is granting a one-year conditional registration under Section 3(c)(7)(C) of FIFRA for the plant-incorporated-protectant, CPG-PPV in C5 HoneySweet Plum.

VI. ACTIONS REQUIRED BY THE APPLICANT

A. Satisfaction of the Conditions of Registration

The applicant must submit acceptable data generated from an independent laboratory validation of the enforcement analytical method within one year of the date the conditional registration is issued.

B. Reporting Requirements

Notwithstanding the information stated in the previous paragraph, it should be clearly understood that certain, specific data are required to be reported to the Agency as a requirement for maintaining the federal registration for a pesticide product. A brief summary of these types of data are listed below.

i. Adverse Effects

Reports of all incidents of adverse effects to the environment must be submitted to the Agency under the provisions stated in FIFRA Section 6(a)(2).

ii. Hypersensitivity Incidents

All incidents of hypersensitivity (including both suspected and confirmed incidents) must be reported to the Agency under the provisions of 40 CFR §158.2140(d).

APPENDIX A: PRODUCT CHARACTERIZATION (40 CFR § 158.2120)

I. Manufacturing Process

Slices of the hypocotyl segment from fresh (or recently stored at 4°C) plum (*Prunus domestica*) cultivar 'BlueByrd' seeds are removed under sterile conditions for transformation. Approximately 8% of slices incubated on selective-differential growth media (50 µg/mL kanamycin and GUS at 37°C) were able to produce shoots and roots following *Agrobacterium*-mediated transformation with binary plasmid pGA482GG/PPV-CP-33. The cloned plum pox virus coat protein was under the PPV-CP-33 plasmid-carried CaMV 35s promoter subcloned into the pGA482GG plasmid HindIII site after restriction enzyme digestion, then transformed to *Agrobacterium tumefaciens* strain C58.Z707 and grown in selective media using kanamycin (50 µg/mL) and gentamycin (50 µg/mL) and tested for GUS activity with an X-Glu solution. Presumptive transformants containing the desired cassette (MRID 471573-01) were confirmed by multiplex PCR for the PPV-CP, nptII and GUS genes as inserted. In all there are 5 mapped inserts into the selected plum event (C5) including one complete and four partial inserts. There is one complete, and one doubled (tail-to-tail) PPV-CP insert (MRID 471573-01). Each PPV-CP insert has a leading 35s CaMV promoter and short untranslated region from the TMV virus in the polycloning site present (MRID 471573-01). Potentially this would produce 3 copies of mRNA for the PPV-CP from the PPV-D strain with an added ATG start codon and TMV leader sequence. Stability was assessed repeatedly from 1990-2005 in propagated transformants and progeny to confirm retention of the inserts, all of which appear to be linked but are an unknown distance from each other on the chromosome.

RNA transcripts were characterized using blots, aimed at the expected 1.4 kb PPV-CP target. Transformants C2, C3 and C4 produced detectible transcripts within 5 hours with blot exposure (32-P hybridization). C5 transcript was barely detected after 40 hours when compared to untransformed controls. No transcript was detected for C6 and no coat protein production was found in either the C5 or C6 events. Analysis of inserts by restriction enzyme digestion and DNA gel blot showed the expected internal PPV-CP BamHI 1.2 kb fragment plus an approximately double-sized fragment from C5. Several EcoRI digest bands besides the expected 7 kb band further indicated multiple, and different, insertions compared to the pGA482GG/PPV-CP-33 plasmid control. In C5 EcoRI bands at 1.9, 3, 5, 7 and 10 kb hybridized with PPV-CP; the 5 and 10 kb bands also hybridize with a probe for nptII and the 7 kb band with uidA. Another 20 kb fragment only hybridized with nptII.

Sequencing of the inserts was accomplished but only about 80% determined "due to sequence repeats, DNA methylation, and the presence of an origin of replication in the insert." The proposed structures (MRID 471573-01) as discussed above are in five parts, one of them complete the others containing duplications or rearrangements and one is listed as an inverted repeat of the PPV coat protein. The mechanism for inversion is not stated though this insert "may be critical for providing PPV resistance."

A. Inert Ingredients

An ampicillin resistance gene (β-lactamase) is present as part of the construct from plasmid pBR322 fragments engineered into plasmid pGA482GG-PPV-CP. However the gene has a bacterial insert containing a cos site (from cosmid MUA10 as derived from pBR322), and is inactivated. Non-functionality of this gene was demonstrated by RNA extraction, and reverse-transcriptase PCR using a reverse primer and spanning the bacterial insert. Analysis of the original plasmid construct but in *Escherichia coli* (*E. coli*) DH5-α using gentamycin to select for the plasmid, and control *E. coli* DH5-α grown in broth but without the plasmid, were plated to LB agar containing 100 mg/L ampicillin. This experiment was replicated twice. Each trial resulted in five colonies of *E. coli* DH5-α containing plasmid pGA482GG-PPV-CP that reverted to functional
β-lactamase by deletion of the cos insert. No spontaneous ampicillin-resistant colonies resulted from *E. coli* DH5-α without the plasmid. Ten C5 plum leaf samples taken from 1997-2005 were assayed for β-lactamase mRNA using reverse primers specific to a 532 bp region spanning the cos site. No mRNA was detected from the samples (archived at -80 ^{o}C) taken at various months throughout those years. Positive controls were the uidA sequence for the GUS transgene, and CAB for the plant chlorophyll A/B – both produced positive PCR results in all 10 samples. Supplemental information provided during USDA's Animal and Plant Health Inspection Service (APHIS) review confirmed by PCR that the cos site remained in the C5 inserted gene. It is evident that even in the highly selective environment of fast-growing *E. coli* cells in presence of ampicillin and harboring a high-copy-number plasmid, that the mutation rate to active amp-R is exceedingly low. None of these conditions is expected to occur for C5 HoneySweet plum trees or fruit.

Other selective marker antibiotic genes present on the original plasmid were tetracycline and gentamycin resistances, present on the part of the plasmid under a bacterial promoter. EPA requested that the applicant provide proof that these genes were absent in the C5 plum trees. On July 10, 2008 the applicant supplied new laboratory tests on extracts of tree fruit and leaves, both the wild-type (BlueByrd) and the PIP (C5). The positive control, plasmid pGA482GG-PPV-CP response is clear, while all lanes of test sample PCR product is negative for the tetracycline and gentamycin resistance genes. PPV-CP DNA is detected from the plasmid and C5 leaves but not from C5 fruit (MRID 474749-02). All genes were absent from the wild-type BlueByrd fruit and leaf samples. The applicant reports that simultaneous tests for nptII, PPV-CP, uidA and 26s rDNA produced requisite PCR products though these results were not shown on these gel photos (page 8, MRID 474749-03).

The gene for nptII (neomycin phosphotransferase II, or kanamycin resistance) is present and does produce both mRNA and protein from the inserted *Agrobacterium tumefaciens* NOS promoter. There are four copies of the nptII gene inserted into the C5 chromosome, of which at least three are thought functional. There is a tolerance exemption for neomycin phosphotransferase II at 40 CFR §174.521. Another inert is the protein from the uidA gene, GUS (E. coli β-D-glucuronidase), under direction of a CaMV 35s promoter. There are two complete copies of the uidA gene on separate inserts, and three fragments on two of the other inserts. GUS has an existing tolerance exemption at 40 CFR § 174.525.

B. Active Ingredient

The PIP active ingredient as inserted is a reverse transcription derivation of the virus coat protein RNA, inserted with a 3' untranslated region with fusion of a start codon and short leader sequence and an *Agrobacterium tumefaciens* NOS terminator under direction of a CaMV 35s promoter. There is one complete copy of the PPV-CP gene, a small fragment of the PPV-CP 35s promoter on another insert and a third insert that is a "3'-3' tail-to-tail copy of the PPV-CP with the 35S promoter for each copy and a portion of GUS sequence flanking each PPV-CP copy. The insert is flanked by plum DNA.

II. Plum Pox Virus Resistance and Mode of Action

The Agency postulates that the C5 honey sweet plum transcribes both a readable (translatable) copy of the mRNA and a complementary copy of that mRNA. These two "complementary" transcripts bind to form a double-stranded RNA molecule that triggers the plant's inherent virus defense mechanism based on post-transcriptional gene silencing (PTGS).

C5 plum was shown to be resistant to the four major serotypes of PPV (Ravelonandro et al. 2001) by a method other than PPV coat protein production in the plant. Subsequent work narrowed the mechanism of resistance to PTGS noting there were low mRNA levels and high methylation of CPG-PPV DNA sequences relating to resistance, upon challenge with PPV (Scorza et al. 2001). C5 trees were selected from testing among five transgenic plums from the same event - C2, C3, C4, C5, C6 - (Scorza et al. 1994) all confirmed by multiplex PCR for the PPV-CP, nptII and GUS genes as inserted (see above). Field experiments in Poland with replicates of each transgenic plum and a wild-type, were chip-bud inoculated in 2 out of 10 replicates, then exposed to natural aphid populations over two years (2003, 2004); as a result, non-inoculated C5 trees were the only ones not infected in the field (Hily et al. 2004). Chip-bud inoculated C5 replicates showed mild symptoms along the chip-bud branches with symptoms decreasing over several more years. Though symptoms had abated, ELISA and IC-RT-PCR (immunocapture reverse transcriptase polymerase chain reaction) testing showed presence of the plum pox virus in a few samples of C5, mainly those still showing symptoms of infection.

The other susceptible transgenic plums and the wild-type (highly susceptible B70146) all developed progressively worsening symptoms. B70146 had readily visible chlorotic symptoms within 1-2 years. Another field trial, in Spain, tested variations of non-transgenic, wild-type plum and fewer of the transgenic plums were tested in Poland. Results confirmed that C5 had durable resistance to inoculated or field-acquired PPV. Even when infected through rootstock, C5 trees were able to keep the spread of the virus in check, and symptoms of the virus were only observed near the site of the graft. Further testing of hybrids and seedlings showed that "the multiple transgene inserts of C5 are closely linked and are transmitted as a single dominant gene (locus)" (Scorza et al. 1998), although PTGS-based resistance in germinated seedlings may be delayed up to a month. In MRID 474749-02, the inverted repeat of the CPG-PPV was further clarified: a piece of the 35s PPV-CP promoter is present on either end followed by a portion of the PPV-CP DNA without the NOS terminator. It is unknown if the sense strand of this insert

can produce mRNA or protein, though as discussed above the antisense strand may produce mRNA from a plant open reading frame.

Table 1: Certified Limits and Nominal Concentrations

Ingredients (CAS number)	PC Code	Purpose	Concentration (% by weight)		
			Nominal	Lower	Upper
Active Ingredient					
Plum Pox Virus Resistance Gene (Plum Pox Viral Coat Protein Gene) DNA	006354	Active ingredient	0.0005	0.00045	0.00055
Inert Ingredients					
Neomycin phosphotrasnferase nptII * CAS number 62213-36-9	806304	Selective marker / Inert ingredient	0.0014	0.0009	0.0015
Beta-Glucuronidase GUS * CAS number 9001-45-0	829082	Differential marker / Inert ingredient	0.0010	0.0009	0.0011

III. Enforcement Analytical Method

As provided in MRID 474749-02, the C5 transformation event of BlueByrd may be differentiated from untransformed trees using the following PCR primers for the inserted coat protein gene:

PPV-CP (1 kbp)
Forward: 5'-AAGCTGACGAAAGAGAGGACGAG-3'
Reverse: 5'-CTACACTCCCCTCACACCGAGGAA-3'
PPV-CP (~70 bp)
Forward: 5'-GCAGGCAAGCCGATTGTAGT-3'
Reverse: 5'-TGTATGACTGGAGGTGGTTGAAGT-3'

The applicant submitted a proposed analytical method based on the use of these primers and a polymerase chain reaction (PCR) technique that should distinguish the transformed C5 HoneySweet plum from non-transformed varieties. The Agency is requiring the applicant to provide data generated by and independent laboratory to validate this method.

APPENDIX B: HUMAN HEALTH ASSESSMENT (40 CFR § 158.2140)

Section 408(c)(2)(A)(i) of the FFDCA allows EPA to establish an exemption from the requirement for a tolerance (the legal limit for a pesticide chemical residue in or on a food) only if EPA determines that the exemption is "safe." Section 408(c)(2)(A)(ii) of the FFDCA defines "safe" to mean that "there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue, including all anticipated dietary exposures and all other exposures for which there is reliable information." This includes exposure through drinking water and in residential settings, but does not include occupational exposure. Pursuant to section 408(c)(2)(B), in establishing or maintaining in effect an exemption from the requirement of a tolerance, EPA must take into account the factors set forth in section 408(b)(2)(C), which require EPA to give special consideration to exposure of infants and children to the pesticide chemical residue in establishing a tolerance and to "ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue... ."

Additionally, section 408(b)(2)(D) of the FFDCA requires that the Agency consider "available information concerning the cumulative effects of a particular pesticide's residues" and "other substances that have a common mechanism of toxicity." EPA performs a number of analyses to determine the risks from aggregate exposure to pesticide residues. First, EPA determines the toxicity of pesticides. Second, EPA examines exposure to the pesticide through food, drinking water, and through other exposures that occur as a result of pesticide use in residential settings.

I. Toxicological Profile

Consistent with section 408(b)(2)(D) of the FFDCA, EPA has reviewed the available scientific data and other relevant information submitted in support of this action and considered its validity, completeness and reliability, and the relationship of this information to human risk. EPA has also considered available information concerning the variability of the sensitivities of major identifiable subgroups of consumers, including infants and children.

A. Data Waivers - Justification

The applicant submitted requests to waive the Tier 1 mammalian toxicity data requirements, except for the requirement to report hypersensitivity incidents, for the registration of the CPG-PPV, listed below. The Agency granted the requests, based on the following rationales.

- There is a long history of consumption of plant virus particles in food without any known toxicity or other deleterious human health effects. Although plant viruses have been found in the intestines (Zhang, et al., 2006), plant viruses have not been shown to replicate in humans and other vertebrates.

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- Non-occupational exposure is minimal to non-existent since the gene is only expressed within plant tissues.

- The C5 plum does not represent a source of new, potentially allergenic or anti-nutrient proteins. Due to the plant's early defense mechanism (PTGS), mRNA transcribed from the CPG-PPV is destroyed. Therefore, it is highly unlikely that any viral coat proteins will be produced in the C5 plum. This conclusion is supported by field expression data submitted by the applicant that found no viral protein

TABLE 2: Tier 1 Mammalian Toxicology Data Requirements and Associated OCSPP Test Guidelines

Acute Oral Toxicity/Pathogenicity	OCSPP 885.3050
Acute Dermal Toxicity	OCSPP 885.3100
Acute Pulmonary Toxicity/Pathogenicity	OCSPP 885.3150
Acute Injection Toxicity/Pathogenicity	OCSPP 885.3200
Hypersensitivity Incidents*	OCSPP 885.3400
Cell Culture	OCSPP 885.3500
Acute Oral Toxicity	OCSPP 870.1100
Acute Dermal Toxicity	OCSPP 870.1200
Acute Inhalation Toxicity	OCSPP 870.1300
Acute Eye Irritation	OCSPP 870.2400
Primary Dermal Irritation	OCSPP 870.2500

* **Hypersensitivity Incidents**: Reporting is required when incidents occur. No hypersensitivity incidents, including immediate-type or delayed-type reactions in humans or animals, during the 18 years that development of the C5 HoneySweet Plum occurred. Should any future hypersensitivity incidents occur, they must be reported to the Agency.

B. Previous Scientific Findings

As part of its consideration of the proposed use of the Coat Protein Gene of Plum Pox Virus as a PIP in the C5 HoneySweet Plum, EPA reviewed previous information regarding the safety of exposure for plant expression of plant virus components, especially the coat proteins. The Agency has previously registered and established food tolerance exemptions for plant virus coat proteins and genes as parts of PIPs. This base of knowledge and experience led to the following three conclusions on which the Agency relied to support a tolerance exemption for the Coat Protein of Plum Pox Virus, in the remote possibility that any currently undetectable residues of this protein are produced in the C5 plum.

i. Plants infected with plant viruses have always been part of food supply without adverse effects.

Virus-infected food plants have always been a part of the human and domestic animal food supply, and components of plant viruses, including plant virus coat proteins (PVCPs), are often found in the produce of many types of crops. For example, at the beginning of this century

virtually every commercial cultivar of potatoes grown in the United States and Europe was infected with at least one potato virus. Even asymptomatic plants are often found to be infected. A common agricultural practice (since the 1920s) involves the intentional inoculation of healthy plants with a mild form of a virus in order to prevent infection by a more virulent form. To date, there have been no reports of adverse health effects in humans or animals associated with consumption of plant viruses in food.

The National Research Council (NRC) observed in its 2000 report that "[h]uman or animal consumption of plants with viral coat proteins is widely considered to be safe, on the basis of common exposure to these types of proteins in non-transgenic types of food." The FIFRA Science Advisory Panel (SAP) also addressed the issue of dietary risk posed by plant viruses and components in December, 1992. In its report from the meeting, the SAP stated that "[s]ince viruses are ubiquitous in the agricultural environment at levels higher than will be present in transgenic plants, and there has been a long history of 'contamination' of the food supply by virus coat protein, there is scientific rationale for exempting transgenic plants expressing virus coat protein from the requirement of a tolerance." The FIFRA SAP again discussed PVCPs on October 11-13, 2004, and "agreed that (because of the human history of consuming virus infected food), unaltered PVCPs do not present new dietary exposures."

Experiments have shown that PVCP levels expressed in plants engineered to resist a virus can be 100-1000 times lower in concentration than in plants naturally infected by the virus. In the C5 plum, no detectable levels of PPV coat protein were found, therefore, if any is produced, it is below the current levels of detection. However, as discussed previously, the C5 plum likely is incapable of producing viral coat protein from the Coat Protein Gene of PPV, since the inserted gene initiates the plant's natural defense mechanism before the protein can be manufactured.

ii. Plant viruses are not infectious to humans and animals.

Any virus/host relationship is characterized by a high degree of specificity. Plant viruses do not infect humans or other vertebrates, and usually only infect plants within a certain taxonomic group. In order to replicate, viruses must insert into the plant genome by means of an insect or other mechanical vector. Plant virus replication depends on components of the viral genome and the plant's ability to transcribe its genes, produce the components and package them into new virus; human and animals lack the corresponding cellular "machinery" for these processes. Thus, it is reasonable to assume that a single component of PPV, the CPG-PPV, will not cause a viral infection in humans and other animals.

iii. Plant viruses are not toxic to humans and animals.

As stated previously, food from crops infected with plant viruses has always been consumed without human or animal toxicity related to these viruses. Additional evidence of the lack of toxicity by a different route of exposure comes from the common practice of injecting laboratory animals with purified plant virus preparations to generate antibodies used for analytical tests (for example, to identify and confirm plant virus infections), without any adverse effects on the animals.

C. Toxicity and Allergenicity Assessment

The Agency is not aware of any PVCPs that have been identified as human food allergens. To determine whether the PPV coat protein or the gene insert would potentially cause toxicity or hypersensitivity, they were sequenced and analyzed for homology with known food allergens and toxins. No alignments that would trigger a concern were identified.

The lack of production of PPV coat protein by the C5 plum, together with reports indicating there have been no hypersensitivity incidents or other adverse effects in researchers handling the trees, fruits and other plant tissues since experimentation with C5 plum began in 1992, support the Agency's conclusion that the CPG-PPV expressed in the C5 plum is safe for human dietary consumption. No further testing, including an *in vitro* digestibility assay, is required.

The safety finding for human consumption of the Coat Protein and the CPG-PPV holds equally for all foods in the stone fruit crop group, and also in almond.

II. Aggregate Exposures

Pursuant to FFDCA section 408(b)(2)(D)(vi), EPA considers available information concerning aggregate exposures from the pesticide residue in food and all other non-occupational exposures, including drinking water from ground water or surface water and exposure through pesticide use in gardens, lawns, or buildings (residential and other indoor uses).

The Agency has considered available information on the aggregate exposure levels of consumers (and major identifiable subgroups of consumers) to the pesticide chemical residue and to other related substances. These considerations included dietary exposure under the proposed tolerance exemption for the Coat Protein of Plum Pox Virus, all other tolerances or exemptions in effect for residues of virus coat proteins and viral coat protein gene PIPs, and non-occupational exposure. Exposure via the skin or inhalation is not likely, since the PIP, which is contained within the genome of the C5 plum plant cells, does not produce PPV coat protein. Further, the C5 plum's resistance to PPV infection reduces or eliminates the production of PPV coat protein following natural infection with the virus. Although the Agency's allergenicity assessment focused on the potential of the Coat Protein of Plum Pox Virus to be a food allergen, the data also indicated a low potential for the PIP to be an allergen. Even if exposure occurred through an unlikely route, such as inhalation, the potential for the Coat Protein of Plum Pox Virus to be an allergen is low, as evidenced by the lack of hypersensitivity in researchers handling C5 plum trees for eighteen years, discussed previously.

Exposure of infants and children to the Coat Protein of Plum Pox Virus from residential or lawn use is not expected, because the use site for the C5 plum is agricultural. In the unlikely event that the C5 plum expresses any PPV coat protein, oral exposure could occur from eating fresh and processed C5 plum products. The level of the coat protein would be extremely low, as indicated by the mode of action of the PIP and the lack of detection of the coat protein in

C5 plums, discussed previously. The same evidence supports the Agency's conclusion that oral exposure from drinking water would be highly unlikely.

III. Cumulative Effects

Section 408(b)(2)(D)(v) of FFDCA requires the Agency, when considering whether to establish, modify, or revoke a tolerance, to consider "available information" concerning the cumulative effects of pesticide residues and "other substances that have a common mechanism of toxicity." These considerations include the cumulative effects of such residues on infants and children. Because there is no indication of mammalian toxicity from CPG-PPV, the Agency concludes that CPG-PPV does not share a common mechanism of toxicity with other substances. Therefore, section 408(b)(2)(D)(v) does not apply.

Determination of Safety for U.S. Population, Infants and Children
FFDCA section 408(b)(2)(C), as amended by the Food Quality Protection Act (FQPA) of 1996, provides that EPA shall assess the available information about consumption patterns among infants and children, special susceptibility of infants and children to pesticide chemical residues and the cumulative effects on infants and children of the residues and other substances with a common mechanism of toxicity. In addition, FFDCA section 408(b)(2)(C) also provides that EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects to account for prenatal and postnatal toxicity and the completeness of the database unless EPA determines that a different margin of safety will be safe for infants and children.

Based on its review and consideration of all of the data and other information submitted by the applicant, in addition to its previous knowledge of plant viruses, including for plant virus coat proteins, EPA concludes that there is a reasonable certainty that no harm will result to the United States population, including infants and children, from aggregate exposure to residues of Coat Protein of Plum Pox Virus. This includes all anticipated dietary exposures and all other exposures for which there is reliable information. The Agency has arrived at this conclusion because the data available on the CPG-PPV demonstrate a lack of toxicity and pathogenicity. The PIP active ingredient, CPG-PPV, is not known to produce any recognized toxins, novel proteins, anti-nutrients, virulence factors or enzymes normally associated with pathogen invasiveness or toxicity in mammals. Thus, there are no threshold effects of concern and, as a result, the Agency has concluded that an additional tenfold margin of safety for infants and children is unnecessary in this instance.

IV. Endocrine Disruptors

As required under FFDCA section 408(p), EPA has developed the Endocrine Disruptor Screening Program (EDSP) to determine whether certain substances (including pesticide active and other ingredients) may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." The EDSP employs a two-tiered approach to making the statutorily required determinations. Tier 1 consists of a battery of 11 screening assays to identify the potential of a

chemical substance to interact with the estrogen, androgen, or thyroid (E, A, or T) hormonal systems. Chemicals that go through Tier 1 screening and are found to have the potential to interact with E, A, or T hormonal systems will proceed to the next stage of the EDSP where EPA will determine which, if any, of the Tier 2 tests are necessary based on the available data. Tier 2 testing is designed to identify any adverse endocrine related effects caused by the substance, and establish a dose-response relationship between the dose and the E, A, or T effect.

Between October 2009 and February 2010, EPA issued test orders/data call-ins for the first group of 67 chemicals, which contains 58 pesticide active ingredients and 9 inert ingredients. This list of chemicals was selected based on the potential for human exposure through pathways such as food and water, residential activity, and certain post-application agricultural scenarios. This list should not be construed as a list of known or likely endocrine disruptors.

The CPG-PPV is not among the group of 58 pesticide active ingredients on the initial list to be screened under the EDSP. Under FFDCA sec. 408(p) the Agency must screen all pesticide chemicals. Accordingly, EPA anticipates issuing future EDSP test orders/data call-ins for all pesticide active ingredients.

For further information on the status of the EDSP, the policies and procedures, the list of 67 chemicals, the test guidelines and the Tier 1 screening battery, please visit our website: http://www.epa.gov/endo/.

APPENDIX C: ENVIRONMENTAL ASSESSMENT (40 CFR § 158.2150)

I. Non-Target Species Effects

All possible and likely effects on mammalian, avian, fish, terrestrial and aquatic invertebrate non-target species were considered. The Agency determined that any hazard to terrestrial or aquatic wildlife when the product is grown is unlikely to exceed the Agency's level of concern (LOC) for non-target organisms. A review of submitted data and published literature showed no evidence of toxicity or pathogenicity from the CPG-PPV or the inert ingredients to wildlife, including those related to federally listed endangered or threatened species, nor any effects on critical habitats, when the PIP is produced in growing plants. No adverse indirect effects are expected from the registered uses of C5 Honeysweet plum trees.

The data requirements for non-target effects are listed in the table below. The OCSPP Guideline refers to the Agency's documents that contain protocols for conducting the studies.

TABLE 3: Non-Target Organisms and Environmental Fate Tier I Data Requirements and Associated OCSPP Test Guidelines

Avian Oral Toxicity	OCSPP 885.4050
Avian Inhalation Toxicity/Pathogenicity	OCSPP 885.4100
Wild Mammal Toxicity/Pathogenicity	OCSPP 885.4150
Freshwater Fish Toxicity/Pathogenicity	OCSPP 885.4200
Freshwater Invertebrate Toxicity/Pathogenicity	OCSPP 885.4240
Estuarine/Marine Fish Testing	OCSPP 885.4280
Estuarine and Marine Invertebrate Testing	
Non-Target Plant Testing	OCSPP 885.4300
Non-Target Insect Testing	OCSPP 885.4340
Honey Bee Testing	OCSPP 885.4380

A. Data Waivers - Justification

Prior to submitting its application for registration, the applicant met with Agency staff, and during the pre-submission meeting it was determined that most of the non-target species data requirements, except for non-target plant testing, were not required for the registration of C5 plum. The decision to waive the data requirements for all non-target organisms is based upon the following justifications

i. Non-Target Plants

The waiver of non-target plant data requirement was requested by the applicant, based upon information from references dating from the 19th-21st centuries on breeding in *Prunus* species. The following summarizes the information that supported the Agency's acceptance of the applicant's requested waiver for conducting a non-target plant test study.

- *P. domestica* is naturally incompatible with most other *Prunus* species. The genomes of native species are mainly diploid, while the genome for C5 is hexaploid
- Forced or artificial hybridization between other *Prunus* species with C5 results in very low percentages of fruit set
- There is a greater tendency of hybrids to be produced when *P. domestica* is the female parent, rather than as a male (pollen-producing) parent
- The extremely low hybridization rate with self-incompatible *P. domestica* ('Honeysweet' is self-incompatible)
- The low vigor of hybrid seedlings
- The low fertility of surviving hybrids

Specifically, viable crosses made with *P. domestica* are unlikely and are often not vigorous or fertile (artificial crosses with success rates up to 1.3% are reported). The only known cross in cultivation, "Alhambra," is three generations removed from an original crossing with *P. domestica*.

The inert ingredients, beta-glucuronidase (GUS) and neomycin phosphotransferase (nptII), have food tolerance exemptions and are not expected to provide any environmental benefit or hazard. While data on the ability of C5 plum to cross with indigenous plum and other *Prunus* species is not readily available or is incomplete, the genome incompatibility (mainly diploid for natives, hexaploid for C5, as stated above), the low fertility of any successful crosses, and the very low frequency of artificial (forced) crosses make this a very small probability. If a successful cross were to occur, in the absence of a PPV infection no benefit or adverse effects from the resistance gene is expected. However, in light of the ongoing invasion of PPV into the U.S. it would likely be beneficial to increase PPV resistance in potential virus hosts, to lower the incidence of virus and of the virus "sinks" (non-agricultural species that are infected with PPV can act as reservoirs for the virus) that enable spread of the virus by aphids.

ii. Other Non-Target Organisms

During a meeting with the applicant prior to submission of the application for registration of the C5 HoneySweet Plum, the Agency determined that sufficient data from peer-reviewed scientific literature, in addition to its own knowledge base (discussed in Appendix B) concerning plant viruses and plant viral coat proteins, would provide justification to waive the requirements of all non-target species testing, except for non-target plants. The following summarizes the information that supported the Agency's acceptance of the applicant's requested waiver for conducting a non-target plant test study.

- There is a long history of consumption of plant viruses (including PVCPs) in foods consumed by animals, including birds, mammals, and other vertebrates, without deleterious effects or evidence of toxicity.
- No exposure to aquatic species (including vertebrate and invertebrate species) is expected since gene is only expressed (as DNA) within the plant genome.
- Exposure to non-target insects, including honey bees, is not expected since the gene is only expressed (as DNA) within the plant genome.
- The gene is incapable of forming infectious virus particles.
- C5 plum is not likely to produce any viral coat protein or other novel protein that would be toxic or infectious to non-target species.
- Even if PPV coat protein is produced in C5 plum, it would be at a very low levels. PPV coat protein and the CPG-PPV sequences have been compared with sequences of known toxins and food allergens, and no sequence matches were found that would raise any concerns.

II. Endangered Species Consideration

Prunus geniculata is the only species of plum listed by the U.S. Fish and Wildlife Service as endangered. This species is found only in patches of remaining scrub areas in parts of Florida, and its level of natural resistance to PPV is unknown. *P. geniculata* is known to thrive after fire, and its habitat is reportedly being deprived of this condition, which has decreased its range and populations. *Prunus domestica* is not found or cultivated in Florida, so *P. geniculata* will not be exposed to C5 plum trees.

Since EPA has determined that no effects are anticipated for any non-target species exposed to CPG-PPV as a result of labeled uses, effects to threatened and endangered species and their designated critical habitats are also not expected. Therefore, a "No Effect" determination is made for direct and indirect effects to listed species and their designated critical habitats resulting from the registered uses of CPG-PPV, as labeled.

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47157301	Braverman, M. (2007) Group A - Product Analysis Test Guidelines: C5 Honeysweet Plum. Project Number: 0377B. Unpublished study prepared by IR-4 Project. 89 p.
47157302	Scorza, R.; Braverman, M. (2007) Plum Pox Viral Coat Protein - Non-Target Plant Waiver Requests. Project Number: 0377B. Unpublished study prepared by Interregional Research Project No. 4. 259 p.
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